

ABSTRACT OF THE DISCLOSURE

Methods and apparatus for evanescent light fluoroimmunoassays are disclosed. The apparatus employs a planar waveguide with an integral semicylindrical lens, and has multi-analyte features and calibration features, along with improved evanescent field intensity. A preferred embodiment of the biosensor and assay method has patches of capture molecules, each specific for a different analyte disposed adjacently within a single reservoir. The capture molecules are immobilized to the patches on the waveguide surface by site-specific coupling of thiol groups on the capture molecules to photo-affinity crosslinkers, which in turn are coupled to the waveguide surface or to a nonspecific binding-resistant coating on the surface. The patches of different antibodies are produced by selectively irradiating a portion of the waveguide surface during the process of coupling the photo-affinity crosslinkers, the selective irradiation involving a mask, a laser light source, or the like.

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